

**In the Specification:**

Please amend the specification as shown:

Please delete the paragraph [0010] and replace it with the following paragraph:

[0010] **Figure 1. Sequence alignment of DPP-IV and POP:** Amino acid sequence alignment of DPP-IV from human (hDPP-IV) (SEQ ID NO: 3) and rat (rDPP-IV (SEQ ID NO: 4), only different residues are shown). The alignment of POP from pork (SEQ ID NO: 5) was performed using structural superposition for the  $\alpha/\beta$ -hydrolase domain only, because of a lack of structural homology for the  $\beta$ -propeller domain. The top line gives additional information about the secondary structure of DPP-IV (yellow arrows and red bars), the glycosylation sites with visible electron density (Y), the potential glycosylation sites (marked in red), the disulphide bonds (green lines between cysteins that are involved) and an arrow that indicates the start of the cloned ectodomain. Sequences are highlighted light gray for the transmembrane part, gray for the part of the  $\beta$ -propeller involved in dimerization, green for residues involved in adenosine deaminase binding, blue for the tyrosine that is involved in the stabilization of the oxyanion of the catalytic intermediate and pink for the catalytic residues.

Please delete the paragraph [0065] and replace it with the following paragraph:

[0065] The ectodomain of DPP-IV, residues 31-766 (sDPP-IV), was amplified by PCR using a cDNA and the oligonucleotides 5'-TGCTGGAATTGGCACAGATGATGCTAC-3' (SEQ ID NO: 6)(with an EcoRI site in bold) and 5'-GCA TGG TAC CTT GAG GTG CTA AG -3'(SEQ ID NO:

**7)** (with a KpnI site in bold). Using the two new restriction sites, the amplified DNA fragment (SEQ ID NO:1) was cloned into pPICZ $\square$ -A vector (Invitrogen) to create a fusion with the  $\square$ -mating factor signal sequence for the secretion of the protein. The use of the EcoRI restriction site added the amino acids glutamine and phenylalanine to the N-terminus of sDPP-IV. The sequence was confirmed by sequencing. pPICZ $\square$ -sDPP-IV was linearized with SacI, transformed by electroporation in *P. pastoris* strain GS115 and the phenotype of the colonies obtained was checked as recommended by the distributor Invitrogen.

Please delete the paragraph [0086] and replace it with the following paragraph:

[0086] The negatively charged oxyanion of the tetrahedral intermediate is stabilized by the main chain NH-group of Tyr631 and by the hydroxy group of Tyr547 (Figure 5). Furthermore, the structure shows that the two Gly628 and Gly632 are important for the formation of the sharp turn to bring the catalytic residue Ser630 in the correct position. This is in accordance with mutagenesis studies on rat DPP-IV (Ogata, S., Misumi, Y., Tsuji, E., Takami, N., Oda, K. & Ikehara, Y. (1992). Identification of the active site residues in dipeptidyl peptidase IV by affinity labeling and site-directed mutagenesis. *Biochemistry* 31, 2582-2587) showing that the sequence Gly<sub>628</sub>-X-Ser<sub>630</sub>-Tyr<sub>631</sub>-Gly<sub>632</sub> (**SEQ ID NO: 8**) is essential for DPP-IV activity.

Please delete the paragraph [0089] and replace it with the following paragraph:

[0089] Essential for substrate binding and catalysis is the N-terminus of the substrates, which has to be unprotected and protonated (Brandt, W., Ludwig, O., Thondorf, I. & Barth, A. (1996). A new mechanism in serine proteases catalysis exhibited by dipeptidyl peptidase IV (DP IV) - Results of PM3 semiempirical thermodynamic studies supported by experimental results. *Eur. J. Biochem.* 236, 109-114). The Diprotin-A complex shows that the terminal -NH<sub>3</sub><sup>+</sup> -group is held very

precisely in position by strong interactions with the carboxylates of Glu205 and Glu206 (Figure 5). A third glutamate, Glu204, stabilizes this substrate recognition site by an hydrogen bonding network with the backbone NH of Arg125, His126 and Ser127 as well as the hydroxy group of Ser127. Importance of the glutamate residues is confirmed by single point mutations that abolish DPP-IV activity (Abbott, C.A., McCaughan, G.W. & Gorrell, M.D. (1999). Two highly conserved glutamic acid residues in the predicted beta propeller domain of dipeptidyl peptidase IV are required for its enzyme activity. *FEBS Lett.* **458**, 278-284). The double Glu-motif is located at the end of an helical segment ( $\square 2^*$  in Figure 1, see also Figure 3) that is highly conserved in the DPP IV-like gene family (Asp-Trp-X-Tyr-Glu-Glu-X)(SEQ ID NO: 9). The helix represents a deviation from the regular  $\square$ -sheet architecture of the  $\square$ -propeller domain (Figures 1 and 3A). The superposition of the active sites of the exopeptidase DPP-IV complexed with Diprotin A and the endopeptidase POP complexed with an octapeptide (Fülöp, V., Szeltner, Z., Renner, V. & Polgar, L. (2001). Structures of prolyl oligopeptidase substrate/inhibitor complexes. Use of inhibitor binding for titration of the catalytic histidine residue. *J. Biol. Chem.* **276**, 1262-1266) shows clear differences. The octapeptide substrate of POP coincides with the double Glu-motif in DPP-IV indicating that this additional structural element functions is very important for substrate selection. Thus, the double Glu-motif is a recognition site for the N-terminus of substrates and restricts the cleavage to dipeptides and the S1 pocket provides an optimal binding to proline and alanine residues leading to a highly specific peptidase.

Please delete the paragraph [0098] and replace it with the following paragraph:

[0098] Mutagenesis studies (Abbott, C.A., McCaughan, G.W., Levy, M.T., Church, W.B. & Gorrell, M.D. (1999). Binding to human dipeptidyl peptidase IV by adenosine deaminase and antibodies that inhibit ligand binding involves overlapping, discontinuous sites on a predicted beta propeller domain. *Eur. J. Biochem.* **266**, 798-810; Dong, R.P., Tachibana, K., Hegen, M., Munakata, Y., Cho, D., Schlossman, S.F. & Morimoto, C. (1997). Determination of adenosine deaminase binding domain

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on CD26 and its immunoregulatory effect on T cell activation. *J. Immunol.* **159**, 6070-6076) identified two important regions in DPP-IV Leu<sub>340</sub>-Val<sub>341</sub>-Ala<sub>342</sub>-Arg<sub>343</sub> (SEQ ID NO: 10) (at the beginning of  $\square\tilde{\square}\square$ ) and Leu294 ( $\square\square$ , at the end of blade 4) and a less important region Glu<sub>332</sub>-Ser<sub>333</sub>-Ser<sub>334</sub>-Gly<sub>335</sub>-Arg<sub>336</sub> (SEQ ID NO: 11) (loop region, at the end of  $\square\tilde{\square}\square$ ) that are all located at the surface of the  $\square$ -propeller domain (Figure 1). Mutation to amino acids found in rat DPP-IV reduces binding affinity to ADA. These residues form a binding site that is located far away from the active site (Figure 2) confirming the independence of DPP-IV activity on ADA binding (Table 1; De Meester, I., Vanham, G., Kestens, L., Vanhoof, G., Bosmans, E., Gigase, P. & Scharpe, S. (1994). Binding of adenosine deaminase to the lymphocyte surface via CD26. *Eur. J. Immunol.* **24**, 566-570). It is concluded that the function of DPP-IV is the localization and orientation of ADA for proper catalysis. The structure gives an indication for the orientation and localization at the cell surface, because the N-terminus must be close to the membrane and the ADA binding would be on the opposite site of the molecule - pointing away from the cell surface (Figure 2). Further, there would be sufficient space enabling interaction of ADA to the A1-adenosine receptor (Ciruela, F., Saura, C., Canela, E.I., Mallol, J., Lluis, C. & Franco, R. (1996). Adenosine deaminase affects ligand-induced signaling by interacting with cell surface adenosine receptors. *FEBS Lett.* **380**, 219-223) which probably plays an important role in the ontogenesis of immune tissues. This view would also support the hypothesis proposing a link for cell-cell interaction via the binding of DPP-IV, ADA and A1-adenosine.

Please delete the paragraph [0104] and replace it with the following paragraph:

[0104] Table 4 lists the atomic structure coordinates for DPP-IV as derived by X-ray diffraction from a crystal of DPP-IV. The polypeptide disclosed in Table 4 is shown in residues 9-736 of SEQ ID NO: 2.